Promoters which initiated with 2 G's (lanes d, e, o) gave lower levels of transcript synthesis in the 4 dNTP reactions, and a promoter which initiated with 1 G (lanes h, i) was not utilized by Y639F in reactions with 4 dNTPs. Within the initially transcribed region, elements other than the number of G's appear to be important. For example, we have found that the w.t. or Y639F polymerases are less efficient in initial transcription extension on the T7 promoter found in the pBS plasmid which initiates GGGC, than on the ϕ10 promoter found in pT75 which initiates with the consensus GGGAGA (data not shown).

Another example is evident in lanes n and o which show the transcripts obtained on a partially single-stranded promoter that initiates GGAAAAUU. Like the promoter used in lanes c and e, this promoter initiates with 2 G's but normal transcript extension on this promoter is less efficient than on the promoter that initiates GGACU. In the 4 rNTP reactions (lanes c, n), the proportion of short transcripts (dimers, trimers) is greater in lane n and we observe significant amounts of poly-rG transcripts beyond the dimer length in lane n but not in lane c. In the 4 dNTP reactions almost all of the transcripts in lane o terminate at the trimer or tetramer length. Because increasing the number of Gs from 1 to 3 enhanced Y639F activity when using dNTPs, we tested a promoter which initiated with a run of 11 G's (lanes p-s). A potential drawback to such a promoter is that such a long run of G's could inhibit the ability of the polymerase to unwind the template. Since T7 promoters with more than 3 consecutive G's in the initial region do not occur naturally, it may be that for other reasons such sequences do not favor initial transcript extension. In fact, we find that this promoter is a poor template. When

it is fully double-stranded, initiation and extension of the transcript is inefficient with either rNTPs (lane p) or dNTPs (lane r), consistent with the expectation that a promoter with this sequence would be difficult to melt. Initiation and transcript extension is enhanced when this promoter is partially single-stranded (lanes q and s), but while poly-G transcripts from 2 to 7 or more bases in length are abundant, runoff transcripts of the expected length are not predominant products. In reactions with 4 rNTPs the transcript patterns of the w.t. and Y639F polymerases are virtually identical so we do not repeat the 4 rNTP reactions with the w.t. enzyme in Fig. 3. Lanes t-z show that the w.t. enzyme is virtually inactive in reactions with 4 dNTPs and the same set of promoter used in lanes b-s.

Relative selectivity of the mutant and w.t. polymerases for dNTPs and rNTPs: Figs. 1-3 present a qualitative analysis of the structure of the transcripts produced by the w.t. and Y639F polymerases with various combinations of NTPs. They show that Y639F can use dNTPs with high efficiency and that both transcript and substrate structure play a role in determining the efficiency of transcript extension. To obtain a quantitative measure of the relative selectivity of the w.t. and mutant polymerases for dNTPs vs. rNTPs under conditions where transcript structure was not a complicating factor, we carried out reactions in which all 4 rNTPs were present but rNTPs or dNTPs were used to radiolabel the transcripts (Table I, see Appendix 1). Under these conditions the unlabeled rNTPs were present in vast excess relative to the labeling NTPs so labeling dNTPs are almost always incorporated adjacent to rNTPs and into transcripts of nearly uniform rNMP structure. On average the mutant enzyme is ~20-fold less selective for rNTPs over

dNTPs than the w.t. enzyme. We used this assay to screen our collection of T7 RNAP active site mutants for increased dNTP utilization. In this screen we looked for increased dATP incorporation in transcription reactions with all of these mutants using supercoiled pT75 as the template, 4 cold rNTPs, and P^{32}-rATP or P^{32}-dATP to label. Since these results were negative with the exception of the tyrosine 639 mutants, we do not present them here, but the mutants tested in this way are listed in "Materials and Methods". It has been reported that use of Mn^{++} instead of Mg^{++} decreases substrate discrimination and increases miscoding for a number of polymerases (Tabor and Richardson, 1989; Nivogi and Feldman, 1981). We, therefore, examined the rNTP/dNTP selectivity of the mutant and w.t. enzymes in Mn^{++}-citrate buffer (Tabor and Richardson, 1989). With Mn^{++} the preference of both the w.t. and Y639F polymerases for rNTPs over dNTPs was markedly reduced.

Relative activity of the w.t. and Y639F polymerases with different NTP combinations: The relative activities of the Y639F and w.t. polymerases with supercoiled pT75 as a template and various combinations of rNTPs/dNTPs were measured in both Mg^{++} and Mn^{++} buffers (Table II, see Appendix 1). In Mg^{++} buffer, substitution of a single rNTP with a dNTP reduces w.t. activity by 20 to >400-fold, but only modestly reduces the activity of Y639F. The rank order of the effect of a particular dNTP substitution on w.t. enzyme activity--dGTP>dATP>dCTP>dTTP=dUTP--matches the order of their addition to the transcript. With the "2 dNTP" reactions the w.t. enzyme was most active when rCTP and rUTP were substituted with dNTPs, corresponding to the 2 nucleotides added latest to the transcript. The w.t. enzyme was inactive with all other "2 dNTP" or "3 dNTP". In the "2